

STEEPING STUDIES ON THE ENDOSPERM FRACTION  
OF MILO SORGHUM

by

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## INTRODUCTION

The high adaptability of grain sorghums to the more arid regions of the plain states has caused the acreage of this grain to become second only to wheat throughout much of Kansas, Oklahoma, Texas, Colorado, and New Mexico. These plantings are much increased in seasons when climatic conditions or parasitical infestation cause abandonment of winter wheat acreage and, at times, have resulted in large surpluses on the market. These factors have led to the extensive research done in this laboratory on the industrial utilization of sorghum grain. This research program has resulted in the development of a dry milling process by which the grain is separated into several fractions which differ in composition. The bran fraction contains most of the wax and fiber of the grain; the germ fraction contains the major part of the oil; and the endosperm fraction, comprising about 70 percent of the whole grain, consists mainly of starch and protein.

Previous research on the wet milling characteristics of the endosperm fraction, and the pasting properties of the separated starch, have shown that while sorghum grits may be milled by the conventional methods, the sorghum starches obtained, especially those of the glutinous varieties, are more sensitive to processing conditions than that from corn (25). The nature of the protein matrix of the sorghum grits, the sulfur dioxide contained in the steep water, and drying conditions were factors which contributed to low yields and adverse paste characteristics of

sorghum starches obtained by the conventional wet milling procedure used for corn. For these reasons, the research was undertaken, using different steeping agents and variations in the steeping conditions.

The goal visualized for these experiments was the establishment of conditions whereby the high yields obtained using sulfur dioxide might be retained without its deleterious effect on the starch granule. A method, standard with respect to laboratory practices, was used in the earlier experiments and the yields obtained with the various steeping agents were related to the yields obtained under the reference conditions in order to evaluate the effectiveness of the differing steeps.

It has been reported previously that the steeping agents appear to act mainly in the capacity of a reducing agent during the softening of the protein binding matter. This softening action has been little understood, though extensively studied, and it was hoped that these experiments might give some knowledge of the mechanism of this action. The experiments using cysteine were undertaken primarily with this goal in mind. An understanding of the chemistry of the softening action in the grain might give means, other than the present, purely empirical, methods, to improve the yield of starch. An improved yield would be helpful in two respects. First, the added quantity of starch recovered would result in improved economics of wet milling, and second, the correspondingly higher protein content of the solid residues would enhance their value as feed adjuncts.

The yields reported in this paper can be related only to the laboratory methods of wet milling. Commercial methods, using larger equipment, should show higher recovery for all agents, although intrinsic relationships of the variations using the differing steep agents should hold true when applied to the commercial methods.

#### REVIEW OF THE LITERATURE

Since the extraction of starch is one of the most ancient chemical processes still practiced, there are voluminous references to it in the chemical literature. The early Greeks used starch and their name for it, Amylon, signifies that it, as contrasted with wheat flour, was prepared without milling. Marcus Cato (9), in a treatise on Roman agriculture, describes the primitive method of starch manufacture. This method consisted of soaking the wheat grains in water, pounding them until they were well crushed, working them into a dough, which then is transferred to a muslin bag. This bag was then kneaded in a vessel containing some water to cause a part of the starch and some of the small particles of gluten to pass through, while most of the gluten and hulls remained in the bag. The suspension then was stirred and passed over a cloth filter to remove more of the gluten, whereupon the filtered starch was recovered by allowing it to settle and decanting the water.

This early process was retained through most of the middle ages and was displaced by the so-called Halle process in which a fermentation step was employed during steeping. The grain was

allowed to ferment for 10 to 20 days at 25° C. Alcoholic fermentation occurred during the early stages, then acid-producing bacteria formed a mixture of acetic, lactic, and butyric acids. In the later stages of the steep, a putrefaction was allowed to proceed, in which was produced rather offensive odors from protein degradation products. The sour liquors were removed after fermentation, and the starch was recovered by washing on a closed washing drum. Yields of 60 percent starch could be obtained by this method, but the recovery of gluten in commercial form was impossible. The offensive odors present in the factory provided an added reason why the process has been abandoned in favor of other methods (17).

The adhesiveness of wheat gluten is a property that is not shared by corn to any great extent and, for this reason, the fermentation process was used for a much longer time in cornstarch manufacture. The first commercial cornstarch factory in the world, at Oswego, New York, used this method to obtain cornstarch as early as 1842 (34).

Calvert (8) states that an alkaline neutralization was used in some factories after the fermented grain had been ground and screened. This process, consisting of six steps, is best described by George Archbold (2): air cleaning to remove dirt, a non-chemical steep at 20° to 60° C. for three to 10 days, a rough grind to break the grain and free the hulls, a screening operation to remove the hulls and unbroken grain, a regrind of the screenings, and finally a treatment with sodium hydroxide in vats; the

starch then was allowed to settle and the gluten decanted.

Calvert (8) also makes reference to some factories in which the sodium hydroxide was added to the steep water, but this practice was not widespread since most plants depended upon fermentation, which would have been inhibited by added sodium hydroxide, for the original softening action.

Maxmillian Toch (36) credits Chiozza for the first use of sulfur dioxide as a steeping agent in cornstarch manufacture. Chiozza operated a small plant of about 200 bushels per day capacity, at Cervignao, Italy, which used a cold water solution of sulfur dioxide as steepant for the corn. The corn was steeped for two to three weeks in large vats containing an approximately 0.2 percent solution of sulfur dioxide, and then was cracked between rollers to break the grain. The cracked grain then was brushed in revolving sieves until only the germ and hull remained on the sieve. The starch and gluten slurry was then tabled to deposit the starch.

The sulfur dioxide steep process of Chiozza was brought to the United States by E. A. Mathiessen (24) in 1880 for use in a small cornstarch factory owned by him. Behr (6) improved the process by using a warm water steep and better cutting assemblies to break the grain. He also discovered that the germ could be floated from a slurry of starch and broken grain if the density of the slurry was held to within 8 to 10 degrees Be. This discovery led to the commercial recovery of the oil which is concentrated in the germ.

As presently practiced by the corn industries, the sulfur dioxide used in the steep is held to about 0.2 percent concentration in the steep water, and the length of steep is generally from 40 to 48 hours, during which time the temperature is usually held to around 50° C.

Due to wide use of the sulfur dioxide steep in the corn industry, it has received extensive study. Kerr (18) states that the steeping process as it is now practiced has the following objectives: to soften the kernel so that the various components of the grain, such as the hull and fiber, germ (which contains nearly all of the oil), gluten, and starch, may be separated from each other with a high degree of efficiency; to remove at least a portion of grain components which are soluble in warm water; to reduce or inhibit the activity of microorganisms which are brought into the mills on the grain; and, to complete the cleaning of the grain.

The softening action of the grain in wet milling is by far the most important step, since the recovery of the starch and by-products depends to a large extent upon the physical condition of the grain when it is sent to the mills. Not very much is known about the mechanism of this softening action, although it has been the subject of much study. Kerr (18) states that hydration is an important factor of the softening action. Cox, MacMasters, and Hilbert (10) have made a microscopical study of changes in the grain which result from the use of sulfur dioxide and several other steepants, at differing temperatures for vari-

ous lengths of time. The first change apparent was the entry of the steep liquor at the chalaza, or basal end of the kernel; subsequently the steep liquor was observed to travel rapidly upward just under the seed coat. It apparently reached the top part of the kernel before starting to penetrate into the aleurone layer to the starch cells of the endosperm. Penetration of the embryo preceded that of the endosperm. At the 0.2 percent sulfur dioxide concentration used in these experiments, less than four hours was required at 49° C. for complete wetting of the germ, and about eight hours for the complete penetration of the endosperm; the latter even then was not softened to any great extent. A method then was used, in the preparation of sectional slides of the grain, which removed the starch, but which left the protein matrix intact and visible. The strands of protein were found to be birefrigent when observed between crossed nichol prisms, indicating that the majority of the molecules in the network are oriented. In crystalline or oriented high polymeric polar substances, it is generally recognized that powerful intermolecular binding forces are operative. As a result, such high polymers are usually strongly knit together and difficult to disperse in solvents. Cox et al. (10) state that this oriented nature of the protein network probably accounts for the toughness of the grain and the difficulty in dispersing, during the milling operation, the protein which surrounds the starch. The protein matrix was found to be little changed by a 24 hour steep in distilled water, and the birefringence was still evident. When sulfur dioxide

was introduced into the steep, however, the protein was found to be altered during the steep, depending upon the time and concentration of the sulfur dioxide. When 0.1 percent sulfur dioxide was used, the matrix was as strong and distinct as ever, but the strands were swollen to some extent. It was appreciably weakened and partially dispersed after a 0.2 percent sulfur dioxide steep. After a 0.4 percent sulfur dioxide steep, most of the protein was dispersed and only a relatively small amount, which showed no birefringence under polarized light, was observed to lie along the cell walls.

Cox et al. (10) further state that the protein matrix in the horny part of the endosperm, just under the aleurone layer does not disperse as readily as does that in the floury part of the endosperm; in the horny endosperm, the extent of hydration is no greater than that occurring in distilled water at any concentration of sulfur dioxide below 0.4 percent.

Cox et al. (10) made further experiments in order to determine whether or not the softening action of sulfur dioxide was due to the acidity of its aqueous solution. Several experiments were run using concentrations of acetic acid and hydrochloric acid equivalent to the sulfur dioxide solutions. It was found that corn steeped in acetic acid was hard to grind, and separation of the starch from the gluten was difficult; only a 70 percent recovery of the starch present in the original grain was obtained, as compared with an 89 percent recovery when sulfur dioxide was used. The corn was much harder to grind after the hydrochloric

acid steep than after that of acetic acid; even though in this case the separation of the starch from the gluten on the table was good, only a 56 percent recovery was obtained. It was found that a sample steeped only four hours in sulfur dioxide was as readily processed as one steeped for 24 hours in the hydrochloric acid solution. A later experiment gave results which indicated that lactic acid has about one-half the softening power of sulfur dioxide, although in the former case, difficulties were experienced in tabling operations.

Cox et al. (10) concluded from the foregoing evidence that the softening action of the sulfur dioxide was probably due, at least in part, to its reducing power. This conclusion is supported in part by the experiments of Olicott, Sapirstein, and Blish (28) who have shown that the sulfites and mercaptans have considerable dispersing powers on wheat glutens. They have shown that reducing agents have a double effect, namely, a chemical action on the proteins and activation of the native papainases present in the grain.

Killinger (19) has discussed the use of sulfur dioxide in relation to its germicidal properties in the steep and he states that it is an effective inhibiting agent for the organisms studied in concentrations down to 0.02 percent, but the free acid reacts to form bisulfites and addition compounds which are not as efficient in this sterilizing action.

Much attention has been given to the study of softening agents other than sulfur dioxide. Sodium benzoate (18) has been

used but some sulfur dioxide must be added if it is to prove effective in the steep. The use of organic halogen compounds, such as monochlorobenzene and o-chlorotoluene, has been patented by Berquist (7). Bartman (5) claimed that the use of formaldehyde was effective, and Eckland (13) recommended the use of ozone.

The earliest alkaline steeping process was patented by Stratton (35), and the process used by Kingsford (20) has been described earlier as the first process of manufacture of corn-starch in America. More recently, attention was again directed to the use of caustic alkalies in the steep (37).

Nash (27) used liquid ammonia, or concentrated ammonium hydroxide, as an agent to remove the protein of wheat flour, leaving the starch in a clean condition.

Fesca (14) recommended the use of a small trace of sulfuric acid in the steep to hasten separation of gluten on the starch table.

Kühl (21) states that fermentation by Lactobacillus lactis acidii, L. delbrueckii, L. bulgaricus, or whey bacteria aids in the separation of the starch.

Riesen et al. (30) have noted that most of the lactic acid producing bacteria, including Lactobacillus delbrueckii, require cystine as an essential part of the culture media, although this is not true of all strains.

Cell permeability may also play a part in the softening of the protein binding matter. According to the present theory of the swelling of protein gels (33), when a dilute solution of an

acid is added to the protein gel there is formed an ionized salt between the protein and the anion of the acid. In the gel the protein ion cannot diffuse away from its fixed position, while, on the contrary, the ions of free acid are freely diffusible in the gel. As a result, a membrane equilibrium is set up between the interior of the gel and the outer solution. In this system there is a greater concentration of osmotically active ions in the gel than in the external liquid. This excess of osmotic pressure leads to a diffusion of water into the gel which gives rise to the swelling of the protein gel. The cohesive forces that tend to hold the gel together supply the mechanism that limits the amount of swelling.

Schmidt (33) also makes the statement that this theory may be applied to any state of subdivision of the protein. This theory may well apply in some modified form to the migration of water, during the steep, into the protein of the native grain. In this case, however, there is present, around the protein binding matter and starch, a cellulose cell wall which must also be penetrated by the liquid.

Davis (11) states that during the period when dormancy is induced in seeds there is a perceptible drop in catalase activity and the respiratory rate of the seed. This investigator also states that the differences observed in germination, after the removal of the seed coat, is very likely due to differences in the permeability of the seed coats to gases during the period in which dormancy was induced. Davis (11) also states that an oxygen

supply to the seed, just below that necessary to cause germination, appears best for the development of dormancy. Several chemicals have been found capable of initiating growth in dormant buds, among these are: ether and chloroform (16); ethylene (31); ethylene chlorohydrin and sodium thiocyanate (13); thioacetamide; hydrogen sulfide, and ethyl mercaptan (26). All of these seem to increase the cell permeability of the dormant seeds.

Hartmann and Hillig (15) advise preliminary digestion of the flour proteins by pepsin in starch analysis by the diastatic digestion method.

Pietz (29) notes that the swelling power of various wheat gluten proteins is related to their sulfur content; proteins possessing the larger swelling powers also have a larger sulfur-nitrogen ratio. This investigator states also that the degradation of proteins in a 0.5 percent papain solution is higher the greater the sulfur content.

Laine (22) has noted that in the enzymatic hydrolysis of zein, a large protein isolated from corn, there occurs a splitting of linkages, probably the disulfide link of cystine, which leads to the appearance of sulfhydryl groups.

Walti (38) first prepared the crystalline proteolytic enzyme ficin from the latex of the fig tree. He states that it has a higher sulfur content than any other crystalline enzyme of protein nature, and that this sulfur is in form of sulfhydryl groups. Ficin was noted to be inactivated by oxidizing agents such as hydrogen peroxide, iodine, oxygen, cystine and phenylhydrazine;

on the other hand it was reactivated with cysteine and sodium thiosulfate.

Rothen (32) states that proteolytic enzymes may operate upon a protein substrate through distances greater than  $100^{\circ}$  A. He then postulates that enzymatic action may originate through a field of forces resulting from the extended resonators according to the theory of London (23).

## EXPERIMENTAL PROCEDURE

### Preparation of Endosperm

The endosperm fraction of Westland milo sorghum, used as raw material in these experiments, was obtained from Grain Products Incorporated of Dodge City, Kansas. This material was produced by a dry milling process and consisted essentially of pure endosperm, with only a small amount of the germ and bran fractions of the grain still retained. The endosperm fraction is termed the grit fraction commercially and will so be referred to throughout the remainder of this paper. Two 100 pound lots of these grits were blended and stored in a covered metal drum which contained an open bottle of carbon disulfide as protection against insect infestation.

### Standard Wet Milling Procedure

Twelve batches of grits were wet milled using an identical procedure to serve as a basis for comparison with the later variations of the steeping conditions. Each steep solution contained

25 ml of chloroform and sufficient distilled water to give a final volume of 1,200 ml. One-kilogram samples of the grits were weighed into clean glass containers and the steep solution added. The containers were then closed, sealed by a rubber gasket, and placed in a constant temperature bath. The temperature of the water bath was then adjusted to 50°, by means of a mercury metastatic thermal regulator, and the sample steeped for 18 hours.

At the end of the 18 hour period, the solution, in each case, was drained from the grits through a 60 mesh wire screen and collected in a clean beaker. The undissolved solids present in this filtrate were allowed to settle to the bottom of the beaker and the liquid was decanted. These recovered solids were returned to the drained grits and the liquid portion was discarded.

The sample of steeped grits was then divided into six equal portions of approximately 200 grams, a suitable size for milling, and each portion placed in a Waring Blender. The glass steep container was washed with tap water and the wash water added to the six portions of grits. Tap water then was used to cover the grits and added until the water level was exactly three inches below the lip of the Waring Blender container; the total volume of the grits plus the added water was 550 ml. Each Blender glass of grits was then placed on a Waring Blender stirring motor and allowed to mill for ten minutes. The Waring Blender was equipped with the sharp edge type cutting assembly which disintegrated the grits by both a cutting and shearing action. The slurry thus

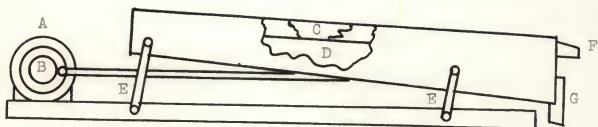
obtained was then screened over the long shaker screen shown in Plate I. This shaker screen was powered by a 110-volt electric motor which agitates it constantly. The screen used was made of 200 mesh stainless steel wire and was provided with a projecting spout which carried any overflow to a large aluminum pan where it was collected to be rescreened. Each portion of cracked grits was poured slowly on the screen and the Waring Blendor container rinsed with approximately 200 ml of water. The tailings on the screen then were washed with approximately 500 ml of water and collected on the aluminum pan by means of a wooden paddle. The tailings from all six portions of cracked grits were divided between two Waring Blendor glasses, water added to within one inch of the lip and reground in the Blendor. These reground tailings were rescreened according to the same procedure as was used for the first screening, and the final tailings from this operation were collected on a tared aluminum pan and dried overnight at 70° C. in a circulating air oven. This fraction was then weighed and designated as the screenings in the data.

After passing through the screen, the slurry, consisting of suspended starch and finely ground gluten, was carried by a trough placed below the screen to a 10 gallon stainless steel stock pot. When the screened slurry from one kilogram of grits had reached the pot, water was added to within six inches of the top. The diluted slurry was agitated with a paddle, allowed to settle for five minutes and then adjusted on a Beckman pH meter to a pH of 5.3 by the addition of acetic acid or ammonium hydroxide.

EXPLANATION OF PLATE I

- A Electric shaker screen motor.
- B Eccentric driving pulley.
- C 200 mesh stainless steel screen.
- D Starch slurry collecting trough.
- E E' Flexible steel supporting rods.
- F Outlet spout for screenings.
- G Outlet spout for starch slurry.

## PLATE I



The stock pot containing the slurry next was placed on a stand at the lower end of the starch table shown in Plate II. The suspension was pumped onto the upper of the two clean, inclined planes which were operated in series. A turbine-type pump circulated the mixture continuously. In order to regulate the rate of flow to a gallon per minute, part of the stream carried by the pump was by-passed into the pot. The agitating effect of this stream flowing into the stock pot helped to keep the starch from settling; this agitation was supplemented by that of an electric stirrer.

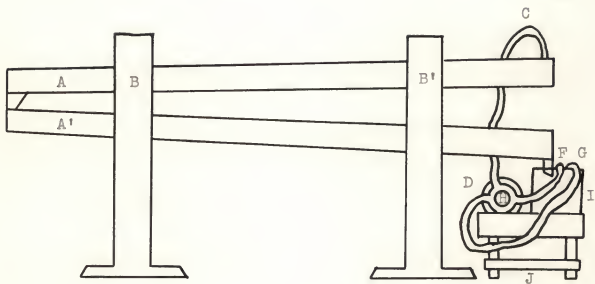
Tabling at the indicated rate of flow was continued for 30 minutes, after which time the circulating pump was disconnected, and the layer of starch was flushed with a gentle stream of tap water to remove the dark film of protein which had settled on the surface. The wash water was collected in the stock pot together with the undeposited portion of the original slurry, and the tables were allowed to drain. The stock pot, containing the resulting mixture, was placed in the outer cold room and left overnight at 4° C. to permit the settling of the undissolved components. At the end of this period, the water was decanted from the solids on the bottom of the stock pot and the solids collected to be dried overnight at 70° C. in the circulatory, hot-air oven. This fraction was weighed and designated in the data as the table solids.

The solidly-packed layer of starch left on the tables was removed and placed in a large Buchner funnel to drain. The table

EXPLANATION OF PLATE II

- A A' Inclined planes, starch tables.
- B B' Wooden supporting beams.
- C Outlet hose at head of table.
- D Electric motor.
- E Starch slurry outlet from tables.
- F Starch slurry intake hose.
- G Starch slurry outlet jet hose.
- H Slurry circulating pump.
- I Steel stock pot.
- J Wooden table.

## PLATE II



was washed free of starch and the washings were collected in a clean battery jar to be added to the starch in the Buchner funnel. The combined starch in the funnel, after it had been freed from water by suction, was washed with tap water several times, and then thoroughly washed with distilled water. After the last washing, the starch cake was crumbled on a large, previously tared, aluminum tray and, with the exception of several lots which were dried in a circulatory air oven at  $70^{\circ}\text{C}.$ , dried at room temperature under a gentle air current from an electric fan.

#### Steeping Agents

Since the primary objective of these experiments was the improvement of the steeping stage of the wet milling process, all operations following the steep were repetitions of the standard experiments which were dried at room temperature.

Experiments were made using a number of substances in variable amounts in the steep water under otherwise standard steep conditions; 25 ml of chloroform was added to the steep water in all experiments. The chemicals employed in this group of experiments were diethyl ether, acrylonitrile, ammonium sulfite, sulfur dioxide, diethylamine, and triethylamine.

It was thought that the lengthening of the steep period from 18 hours to 48 hours, while keeping the temperature constant at  $50^{\circ}$ , might aid in the softening of the grain. Experiments were run at the 48 hour period using lactic acid, acrylic acid, ammonium oleate, and thioacetamide. For purposes of comparison,

one batch of grits was steeped in the standard chloroform solution also for 48 hours with no appreciable increase in starch yield over that of the 18 hour steep.

In order to determine if the temperature of the steep influenced the yield in these experiments to any extent, several steeps were run using the standard chloroform solution at temperatures of 37°, 63°, 65°, and 67° C. for 48 hours and for 18 hours. The results of these experiments showed no significant variations in yield, although the starch quality often was adversely affected. Experiments also were run at the different temperatures using the following agents; sodium salicylate, versine, potassium periodate, sodium bisulfite, ammonium sulfite, and sulfur dioxide. These experiments likewise gave no significant changes in the yield of starch with the exception of sulfur dioxide which gave a noticeably larger yield than the other steeping agents named. The pasting properties of the corresponding starches were all adversely affected by the high temperatures, as well as by the presence of the majority of the steeping agents.

The low yield of starch and the inferior pasting qualities of the starches obtained in the foregoing experiments led to the use of enzymes in the steep solutions. Several previous experimenters (18, 10) have postulated that some of the softening power of sulfur dioxide in the steep solution might be due to the activation of the papainases already present in the grain. Thus, it was thought possible that the use of proteolytic enzymes which were not native to the grain might also give the same effect.

Experiments were run using pepsin and ficin at various concentrations, temperatures, and lengths of steep time. Pepsin proved to have no appreciable effect at the concentrations used, but ficin gave relatively high yields of starch at all temperatures when used at concentrations of 0.1 percent or greater.

Several experiments next were run using 0.05 percent ficin, with a varied quantity of sodium thiosulfate added to the steep water for the purpose of keeping the ficin activated throughout the whole steep time. The high yield of starch obtained using one gram of sodium thiosulfate led to the use of cysteine hydrochloride as the activating agent in several experiments.

Several experiments also were made in which cysteine hydrochloride alone was used as the steepant; in addition cysteine hydrochloride was used after a preliminary steep in various acids. It was thought that some understanding of the mechanism of the steep process would be forthcoming from these latter experiments involving cysteine hydrochloride.

### Viscosity

Paste viscosity records were determined for most of the starches obtained in these experiments on a rotating cylinder viscometer that was built in this laboratory.<sup>1</sup> This instrument measures continuously the changes in viscosity which take place over a temperature cycle between room temperature and 93° C. (4).

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<sup>1</sup> Paste viscosity records were made by Mrs. Rhoda McIntyre.

The following procedure was used to determine the viscosity records of the various starches. Forty grams of starch, corrected for moisture, and 360 grams of water, including that present in the starch, were placed in the rotating cup, a temperature of  $20^{\circ}$  C. established, and the suspension heated at a rate of  $0.5^{\circ}$  per minute to  $93^{\circ}$  C. This operation is called the heating period. The temperature was held at  $93^{\circ}$  for a 30 minute cooking period, after which the paste was cooled at a rate of  $0.5^{\circ}$  per minute to the temperature at which the low temperature peak developed, usually at about  $24^{\circ}$  C. The paste then was cooled for about 10 minutes after the cold peak was reached in order to make sure that the viscosity reached was actually the maximum. The angular velocity of the rotating cup was maintained at 60 r.p.m. Torque measurements, expressed in grams, were made with sufficient frequency to permit smooth curves to be drawn. Two maxima developed in the viscosity records, one in the heating period ( $W_1$ ) and the other near the end of the cooling period ( $W_2$ ). The ratio ( $W_1/W_2$ ) is used as an empirical index of the starch quality (3).

The rotation of the cup was stopped at the end of the cooling period, and the paste allowed to remain undisturbed for about three hours. At the end of this period, a small bucket was attached through a cord and pulley system to the suspended torque cylinder. Small steel shot were allowed to pour at a constant rate into the bucket until the gel broke. The weight in grams required to produce movement of the suspended cylinder was re-

ferred to as the gel strength (G.S.) (3). The quantity  $[\overline{\text{G.S.}} - W_2]/(W_2) \times 100$  is indicative of the relative length of the starch paste, since the longer starch pastes have higher numbers for this quantity; for this reason it is tentatively called the gel index.<sup>1</sup>

From more than 50 measurements of corn and sorghum starches made in this laboratory, it was found that the errors of measurement of  $W_1$ ,  $W_2$ ,  $W_2/W_1$ , and G.S. were within 2, 5, 4, and 13 per cent, respectively (3).

### Feeding Stuff Analysis

Wet milling fractions collected in several selected experiments were analyzed<sup>2</sup> for moisture, ash, nitrogen (protein), crude fat, crude fiber, and starch by the A. O. A. C. methods (1).

### RESULTS AND DISCUSSION

#### Starch Yields from Standard Chloroform Steeps

The wet milling procedure, adopted as standard in this laboratory, proved to be capable of fairly close reproductability, as was shown in Table 1. The average deviation was 25.5 parts per thousand. Several of the experiments showed deviations over 50 parts per thousand in this series. These larger deviations may be accounted for by the fact that, in this early series of

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<sup>1</sup> Personal communication of unpublished data by Dr. H. N. Barham.

<sup>2</sup> Analyses were performed by the State Board Laboratory of the Kansas State Agricultural Experiment Station.

Table 1. Yields of fractions and starch yield deviations, calculated on a dry basis.  
Steeping conditions: 18 hours at 50° C. Steeping agent: chloroform.

Sample number	: Yield :		: Loss* :		: Yield of :		: Starch yields :	
	: screenings :	: solids :	: percent :		: starch :		: Actual :	: Deviations :
							: percent :	: in :
								pts./1000
184- 2	9.90				58.50	1.66		28
3	10.75				61.50	1.34		22
5	10.70				60.05	0.11		2
12	14.73	18.03		7.6	59.64	0.52		8
13	13.03	15.91		9.2	61.94	1.79		29
15	15.34	12.81		8.1	64.72	4.46		68
16	15.60	10.70		15.8	57.90	2.26		39
17	14.50	16.50		9.6	60.40	0.24		4
18	15.20	13.50		10.2	61.20	1.04		17
19	12.94	20.16		6.4	60.59	0.33		5
20	17.15	17.75		8.3	56.80	3.36		59
21	17.08	20.16		9.1	59.76	1.40		23
Average	13.19	16.17		9.7	60.16	1.55		25.5

\* Losses from various sources: solubles and suspended material from steep and table waters.

Table 2. Effects of time and temperature of steep. Yield of fractions calculated on a dry basis. Steeping agent: chloroform.

Sample number	:Temperature :of steep : ° C.	:Length : of steep : hours	:Yield of		: Loss*	: Yield of : starch
			:Yield of	:table		
			:screenings	: solids		
Percent						
184- 58	50	48	13.70	17.05	10.40	58.83
105	37	48	10.70	21.90	4.80	62.50
65	63	48	13.80	20.06	8.40	57.70
69	65	48	13.20	13.00	17.50	56.30
71	67	48	13.80	28.60	5.50	56.10
76	67	18	18.60	9.90	13.80	57.70

\* Losses from various sources: solubles and suspended materials from the steep and table waters.

twelve experiments, the Waring Blenders used were excessively worn and may have given unequal grinding action on the different samples. It is obvious, however, that due to the lengthy procedure for these experiments, the results probably are capable of no closer reproductability than that shown.

The table solids were not collected in the first three experiments in this series, for which reason the amount of losses could not be calculated. The losses in this series show about the same deviations as were shown by the starch yields; the largest spread being that between the 6.4 percent and the 15 percent loss. It is apparent from the drop in the table solids yield for the two experiments 184-16 and 184-18 that these large losses were probably the result of some of the solids being carried off as suspensoids in the decanted water. The screenings also varied approximately the same as did the starch yields. There appears to be a qualitative inverse relationship between the size of the starch yield and the screening yield, although this is modified to some extent by the amount of the table solids collected. Using this series as a basis for comparison, it is obvious that yields can be expected to vary between 57 percent and 62 percent because of the uncontrolled variables which participate in the wet milling process.

### Yields with Varying Steep Conditions

The data listed in Table 2 seem to indicate no relationship between starch yields and the temperature and length of steep. All the yields reported fall well within the bounds of the variations exhibited by the twelve standard chloroform experiments. Other investigators (10) have found that changes in the temperature and length of steep do affect the quality of the protein, but could find no significant variation in starch yield due to these factors. These investigators have also found that a period of eight hours is necessary for the water to penetrate into the endosperm before any softening action occurs. This period would be much shortened in the experiments described in this paper because only the scalped endosperm fraction was used as a raw material, in place of the whole corn used by Cox et al. (10). Since all experiments noted in this paper were steeped for over 18 hours, and the rate of hydration of the protein is known to become progressively slower at longer steep times, it would be expected therefore, that there would be little variation in starch yields obtained using differing lengths of steep time.

### Yields Obtained Using Chemical Steepants

The yields obtained in experiments given in Table 3 also showed little variation from the limits set by the twelve standard chloroform experiments. The yield obtained in the experiment employing ammonium sulfite, 184-42, was highest within

Table 3. Effects of various steeping agents on yields of fractions. Yields of fractions calculated on a dry basis. Steeping conditions: 18 hours at 50° C.

Sample number	Steeping agent used	Amount of :steeping agent used:		Yield of : screen- : solids :		Yield of : starch :		Loss*
		gms	gms	gms	gms	gms	gms	
184- 23	diethyl ether	20.0	15.20	13.40	62.50	8.90		
37	acrylonitrile	20.0	15.10	14.21	61.59	8.10		
41	ammonium sulfite	4.2	13.21	17.00	60.01	9.80		
42	ammonium sulfite	4.2	15.54	12.51	64.90	7.00		
43	ammonium sulfite	4.2	11.70	19.20	61.60	7.50		
44	ethylene	5.0	14.30	21.30	57.20	7.20		
46	ethylene	5.0	13.20	20.00	59.50	8.30		
50	lactic acid	5.0	11.45	29.21	56.20	3.10		
51	sodium thiosulfate	2.0	10.20	18.30	57.19	14.31		
63	glyceryl oleate	2.0	12.95	15.60	62.50	10.95		
100	lactic acid	4.0	13.50	25.70	55.80	5.00		
102	sulfur dioxide	2.0	24.60	9.3	60.80	5.30		

\* Losses from various sources: solubles and suspended material from the steep and table waters.

this group of experiments. The added starch yield seemed to come from the tabling operation, as was deduced from the fact that the table solids were very low while the losses remained constant. This conclusion was supported by the yields obtained in experiments No. 184-41 and 184-43, which were intended to be duplicates of 184-42.

The sulfur dioxide, used in experiment No. 184-102, appeared to have very little action in this temperature range and length of steep. The yield of screenings was much higher, however, with sulfur dioxide under these conditions, and the table solids were proportionately lowered. This might tend to show that, though the starch has been removed, the protein has been retained in the cell walls of the screenings. This is highly desirable because the recovery of this protein is relatively much easier from the gross screenings, which can be recovered by a simple screening action, than from the table solids, which must be isolated, by some means, from a large amount of water. Experiments No. 184-23, 184-37, 184-44, and 184-46 were run with a view of determining whether the reported ability of these reagents to increase the cell permeability of the grain (16, 31) might not be a factor in increasing the yield. Experiments No. 184-56 and 184-54, reported in Table 4, also were run with the same purpose in mind. It is apparent from the yields obtained that this factor does not alone predetermine satisfactory steeping. The experiments utilizing ammonium sulfite, Nos. 184-41, 184-42, and 184-43, and sodium thiosulfate, 184-51, were run to determine whether these

Table 4. Effects of various steeping agents on yields of fractions. Yields of fractions calculated on a dry basis. Steeping conditions: 48 hours at variable temperatures.

Sample number	Steeping agent used	Amount of agent used : gms	Temperature of steeping : ° C.	Yield of :				Loss %			
				screenings	solids	tablets	starch	solids	starch	tablets	starch
184-57	lactic acid	4	50	18.10	17.20	55.40	8.00				
56	acrylic acid	2	50	11.29	22.00	55.73	11.00				
62	ammonium oleate	2	50	13.50	17.30	62.00	13.60				
70	sodium salicylate	2	65	12.80	28.80	55.80	2.60				
81	versine	5	67	11.80	17.20	66.50	5.51				
83	potassium periodate	2	67	21.73	12.80	53.20	6.83				
87	sodium bisulfite	12	67	10.80	22.00	62.70	4.50				
92	ammonium sulfite	5	67	13.00	15.60	64.00	8.40				

Table 4. (concl.)

Sample number	Steeping agent used	Amount of agent used : gms	Temperature of steeping : °C.	Yield of screenings	Yield of solids	Yield of starch	Loss*
					Percent		
184-93	diethylamine	5	67	17.90	16.50	47.00	18.20
97	triethylamine	5	67	25.40	12.80	54.00	17.80
95	sulfur dioxide	2	67	8.70	18.30	66.50	6.50
96	sulfur dioxide	2	67	7.40	22.60	65.60	4.40
54	thioacetamide	2	50	11.30	16.80	54.00	8.10
110	sulphosalicylic acid	2	50	13.70	19.20	54.90	11.60

\* Losses from various sources: solubles and suspended material from steep and table waters.

might be the active agents in steepers to which sulfur dioxide has been added. It can be seen that these and the others listed in Table 3 had little effect.

Most of the yields obtained using different steepants under differing conditions, reported in Table 4, were very nearly within the range established by the standard chloroform experiments. Experiments No. 184-95 and 184-96, however, employing sulfur dioxide as a steepant, showed a definite increase in yield over most of the other steepants. This was accomplished at the expense of the screenings, as may be inferred from the markedly reduced screening fraction. The table solids fraction in both of these experiments were somewhat above what might be expected, therefore we may assume that the protein, in this case, is more dispersed than in the lower temperature sulfur dioxide steep. The experiment using diethylamine, 184-94, and triethylamine, 184-97, show much lower yields than the rest of the experiments. Both of these experiments also show a very high loss. These reagents seem to cause much of the protein to be solubilized, as well as to cause the protein lattice to break in such a manner as to leave much of the starch still associated with the protein; this action causes the unreleased starch to be carried over the table. None of these reagents showed sufficient influence on the starch yield to be noteworthy, or appeared worthy of further research. It may be noted, however, that the yield with versine was also very high but the characteristics of the starch isolated in this experiment were inferior; the starch isolated was so affected that its entire viscosity curve was greatly lowered.

Table 5. Effects of enzyme steeping agents, in different concentrations, upon yields of fractions. Yields calculated on dry basis. Steeping conditions: variable time and temperature.

Sample number	Amount of enzyme used : gms	Tempera- : ture of steep : ° C.	Length : of steep : hours	Yield of : screen-ings : %	Yield of :			Loss*
					table :	solids :	starch :	
					Percent			
Ficin								
184- 84	1.0	67	48	4.98	11.50	74.33		9.40
86	1.0	67	48	6.28	12.10	75.40		6.60
98	2.0	67	18	5.30	13.90	72.40		8.50
82	0.5	67	48	6.98	20.01	65.50		7.50
99	1.0	50	48	5.10	15.10	73.60		6.20
106	1.0	37	48	6.00	17.30	68.20		8.50
107	1.0	37	48	5.61	16.10	69.10		9.20
Pepsin								
103	1.0	50	48	15.40	19.00	61.20		4.40
104	1.0	50	48	13.40	21.50	60.40		4.70

\* Losses from various sources: solubles and suspended material from steep and table waters.

### Yields Obtained in Enzyme Steeps

Failure in the previous work to obtain sizable increases in starch yields by employing numerous steepants, together with reported effects by enzymes (28), led to the use of enzymes as steeping agents. Results with the use of ficin were uniformly good (Table 5). The yields in experiments No. 184-84 and 184-86 were the highest of the whole series. All of the experiments showed a very much increased yield with the exception of 184-82, which had only a 0.05 percent concentration of the ficin present in the steep water. All experiments showed low screening yields and low losses, the largest variation being the amount of table solids. It is to be noted that the low-yielding 0.05 percent experiment had a much higher yield of table solids, the screenings and losses remaining about the same. This may be interpreted as meaning that the effectiveness of starch recovery from the table is the most important factor in determining the starch yield in this series of experiments, since the protein is apparently disrupted enough that it can pass through the screen about equally in all experiments. This action possibly may be the result of some mechanism whereby the protein may be attacked first at the point where it is attached to the cellulose cell walls, and then attacked farther down the chain to break the protein matrix enough to permit escape of some of the starch from the network. This type of action would then produce a mass of protein material, holding starch still trapped within it, which

Table 6. Effects of supplementary activators, plus ficin, on yields. Yields calculated on a dry basis. Steeping conditions: 48 hours at 50° C.

Sample number	Added chemical agent	Amount of chemical agent	Yield of screenings	Yield of table solids		Yield of starch	Loss*
				:	:		
		gms		Percent			
184-108	sodium thiosulfate	10	5.10	25.40	63.60	8.9	
109	sodium thiosulfate	1	6.20	19.60	69.60	5.6	
114	cysteine hydrochloride	2	5.20	16.20	71.50	7.1	
115	cysteine hydrochloride	2	5.10	16.20	72.80	5.9	

\* Losses from various sources: solubles and suspended material from steep and table water.

would then pass over the table without being deposited. The extent of further breakage would thus be the measure of the amount of starch deposited on the table. The results in this series seemed remarkably uniform in following a direct relationship with the temperature of the steep and, within limits, the amount of ficin in the steep water. This might be accounted for by the fact that, at these higher yields, the changes occurring during the steep have reached a point where continued modification of the protein slowly approaches a limiting value; thus perhaps little additional starch can be obtained by further increase in temperature and concentration of steepant.

The experiments with pepsin as a steepant showed no appreciable increase in starch yield. This might be expected since pepsin is more specific than ficin in its action on proteins. Here again, it will be noted that the screenings have returned to those levels obtained in the standard chloroform steeps.

The present experiments with ficin, and enzymatic behavior previously reported (38), led to the work recorded in Table 6. It was hoped that steep solutions, utilizing a smaller amount of ficin along with some enzyme activator, would provide protein modification equal to that obtained using larger amounts of the enzyme alone. Experiment No. 184-108, utilizing a solution of one percent sodium thiosulfate plus 0.05 percent ficin, did not give as high a yield as the experiment using 0.05 percent ficin alone. There is reason to believe that this is due to the large excess of the sodium thiosulfate; experiment No. 184-109, using

only 0.1 percent sodium thiosulfate plus 0.05 percent ficin, gave a yield over four percent higher than with 0.05 percent ficin alone.

The last two experiments of Table 6 employed cysteine hydrochloride as the activator. The yields of 71.5 and 72.8 percent obtained in these experiments compare very favorably with that of 73.6 percent obtained using one gram of ficin alone under similar conditions. It is apparent from the foregoing data that the cysteine hydrochloride is a good activator for the enzyme. This fact is explained by experiments which have shown that the action of ficin consists, in a large part, of breaking the disulfide link of proteins (38). The sulfur present in ficin is in form of sulfhydryl groups in the activated form and is deactivated by cystine, which contains the disulfide link. This disulfide link is considered to be one of the weaker bonds present in proteins, in spite of the fact that it is often an important structural link of the native proteins. It has been noted previously that, in the hydrolysis of zein by enzymes, there occurs a splitting of the disulfide link of cystine which leads to the appearance of sulfhydryl groups in the hydrolyzate (22). As this might occur in situ as well as in the isolated protein, we were led to postulate a possible mechanism for the softening action of sulfur dioxide and the enzymes reported in this paper.

The first step in this postulated mechanism might well involve a preliminary swelling, and a decrease in steric hindrance. The first step could be brought about in one or more ways. With

sulfur dioxide as the steepant, the ions present could permit the steep solution to enter the protein and deform the structure. Or, with the enzyme present, it might be due to the field of forces Rothen (32) postulates as being the primary action of enzymes. This preliminary swelling of the protein might well expose the site of the disulfide link, which in native proteins is recognized to be fairly well protected, for metathetical reaction with the sulfhydryl groups of ficin, cysteine hydrochloride, or other mercaptans. The action of reducing agents is also explained on this basis as the disulfide link may be broken by an oxidation-reduction reaction. The experiments of Olcott et al. (28), in which reducing agents were found to give an actual dispersing effect on wheat gluten, were performed on a dispersed gluten in a solution of the reducing agent and water.

The action of the lactic acid bacteria might also tie in with this postulated mechanism. Riesen et al. (30) have noted that several of the strains of these bacteria, which were reported by Kuhl (21) to aid in causing separation of the starch from protein, require cystine as an essential part of their culture media.

The experiments listed in Table 7 show the results of attempts to obtain increased yields without the presence of sulfur dioxide or ficin. These gave somewhat increased yields over those of most of the standard series of experiments. The experiment involving a preliminary steep with lactic acid gave the largest starch yield, 68.50 percent. This is well above even the

Table 7. Effects of preliminary steep, followed by the use of cysteine hydrochloride. Yields calculated on a dry basis. Steeping conditions: a preliminary steep of 18 hours followed by another of 48 hours; all steeps at 50° C.

Sample number	Preliminary steeping agent added	Amount : preliminary : inary : steep : agent : gms	Amount : cysteine : hydro- : chloride : gms	Yield of : screen- : ings	Yield of : table : solids	Yield of : starch	Loss*	
					Percent			
184-111	none	2	11.10	18.90	65.60		4.40	
112	acetic acid	2	1	18.30	14.90	64.30	2.50	
113	lactic acid	2	1	16.80	10.70	68.50	4.00	

\* Losses from various sources: solubles and suspended material from steep and table water.

highest yielding standard experiment. The starch yield obtained in experiment No. 184-111 using no preliminary acid steep and 0.2 percent cysteine hydrochloride gave the next highest yield of starch, 65.60 percent. No. 184-112, using acetic acid, gave the lowest yield of starch, 64.30 percent. The screenings yield was somewhat higher in both the experiments involving a preliminary acid steep than in the experiment using only cysteine hydrochloride, while the table solids yields were lower. This might tend to show that the cysteine hydrochloride can react with the protein near the cell wall, as was postulated for the amine steeps, but must have preliminary distortion of the protein to react further down the chain. It seems certain that the cell permeability plays a part in this action.

#### Results of Feeding Stuff Analysis

The results of the complete feeding stuff analysis are listed in Table 8. The starch obtained in the ficin experiment, 184-84, was fairly pure with respect to contaminants such as protein. The starch listed as 184-71, steeped in chloroform, was rather high in protein although the reason for this was not readily apparent. It was interesting to note the rise in crude fiber in the screening fraction in comparison with increasing starch yield. In experiments No. 184-5 and 184-71, the screenings fraction ran 6.36 percent, 5.22 percent crude fiber, respectively. For the ficin run, 184-84, the screenings were 15.12 percent crude fiber, and the starch yield was 74.33 percent. It seems

Table 8. Correlation of yields of fractions and complete feeding stuff analyses of two standard wet milling experiments with those obtained from one in which ficin was used as a steepant.

Fraction :	Yield :	Protein :	Ether : extract :	Crude : fiber :	Ash :	N-free : extract :	Carbo- hydrates
				Percent			
grits	7.85	1.42	1.06	0.67	88.90	89.96	
	Original 184 grits						
	184-5 Chloroform steeped at 50° C. for 18 hours						
starch	60.05	0.49	0.30	0.15	0.12	98.75	98.90
screenings	10.70	19.12	1.39	6.36	0.52	73.00	73.37
	184-71 Chloroform steeped at 67° C. for 48 hours						
starch	56.10	1.02	0.12	0.14	0.25	98.45	98.59
screenings	13.80	18.39	1.69	5.22	0.58	74.00	79.20
table solids	28.60	19.05	1.19	1.67	0.50	78.10	78.60
	184-84 Ficin, steeped at 67° C. for 48 hours						
starch	74.33	0.69	0.10	0.13	0.13	98.90	99.03
screenings	4.98	8.00	4.34	15.12	1.54	71.00	72.54
table solids	11.50	25.62	2.16	1.14	0.54	70.09	71.32

clear that very little of the fiber went through the screen relative to the increased starch yield. This was emphasized also by the fact that the table solids ran only about 1.5 percent crude fiber in both the chloroform steep experiment and the ficin steep experiment. This crude fiber was probably the cell walls of the grits and, as such, resisted disintegration. The cell walls may have been the limiting factor postulated earlier as the cause of the close correlation of the ficin steep series. The fact that the table solids were high in protein even in the ficin experiments shows that, in spite of the high yield of starch, it might be possible to improve this operation, since as stated previously, the best location of the proteins, with respect to industrial processing, is in the screenings.

#### Viscosity Records

Viscosity records of the starch pastes were determined for selected experiments, in the rotating cylinder viscometer built in this laboratory (4). The term, viscosity record, is used here to represent the viscosity recorded by the instrument when 10 percent starch suspensions were carried, at a constant rate of shear (60 r.p.m.) from 20° C. through gelatinization to 95°, through cooking at 95°, and through cooling to the temperature corresponding to the maximum viscosity of the cold paste (3). The maximum viscosity observed during the heating cycle,  $W_1$ , appears to be a measure of the availability of the starch granules to water during gelatinization under the conditions employed; thus any treatment

which lowers  $W_1$ , or displaces it towards a higher temperature, may be said to have decreased the amount of water available to the granule within a given time, or to have caused a higher energy requirement for the starch-water reaction (3). The paste index,  $W_2/W_1$ , is a qualitative measure of the glutinous character of the starch paste; the lower the paste index, the greater the fluidity of the cold paste. The empirical term gel index,  $[T(G.S.-W_2)/W_2 \times 100]$ , is a rough measure of the relative length of the starch paste; the higher this index is, the longer is the starch paste.

The two sample viscosity graphs have been included for the purpose of illustrating some of the effects of the conditions of steeping on the paste characteristics of starches. The two starches, steeped at  $37^\circ$  and  $67^\circ$ , graphed in Fig. 1, illustrate the effect of temperature on the starches. The one steeped at the lower temperature, 184-105, may be observed to possess a higher hot peak, paste index, and gel index than the one steeped at the higher temperature. The effect of the lower temperature is, in the case of these samples, to increase the pasting capacity, glutinous characteristics, and length of the starch pastes. These results hold true for all curves of experiments run at differing temperatures. The whole curve before the cooking period, for the starch steeped at  $67^\circ$ , is observed to have shifted toward higher temperatures, compared with that steeped at  $37^\circ$ . This behavior reflects the tendency of starch granules to progress toward a limiting structure when environmental conditions, such as higher temperatures, permit. This limiting

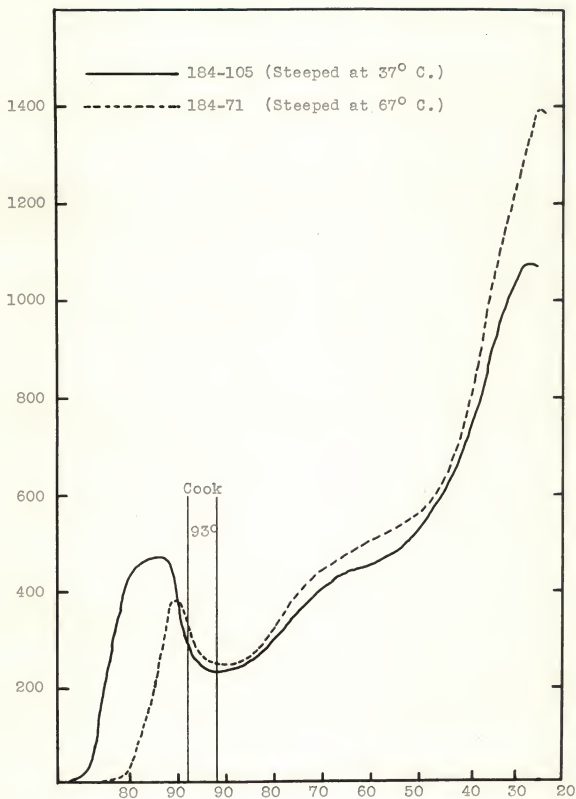


Fig. 1. Effects of the temperature of steep on the viscosity of starch pastes.

structure seems to possess such a configuration that it requires a higher energy consumption for the starch-water reaction to proceed.

The curves in Fig. 2 were plotted to show some of the differences observed using different steepants at 67° C. All three of the curves are noted to be shifted towards the higher temperatures because of the 67° steep, as noted previously.  $W_1$  is highest for the starch steeped in chloroform; it has an intermediate value for the ficin steep, and shows the lowest value for the sulfur dioxide steep. The paste index,  $W_2/W_1$ , will be observed to follow in the same order, 3.66, 2.68, and 2.65. Thus the loss of pasting capacity due to an elevated temperature is likewise influenced by the steepant used. The gel index, however, is in the reverse order; 57.2 for the chloroform, 114.2 for the ficin, 128.3 for the sulfur dioxide. It may be seen from the foregoing data that the ficin and sulfur dioxide tend to make a longer starch, but one with a lower cold fluidity.

The data listed in Table 9 show several sources of variation. The first noted was due to changes in the method of drying of starches. The oven-dried starches have a  $W_1$  about equal to that of the air-dried starches, with the exception of 184-3, which is much lowered. The lowering of  $W_1$ , in this case, cannot be accounted for unless it is due to the fact that this sample remained in the oven longer than the others (over the week-end). The paste indices for the oven-dried starches appear to be somewhat above that observed for the air-dried starches milled from grits

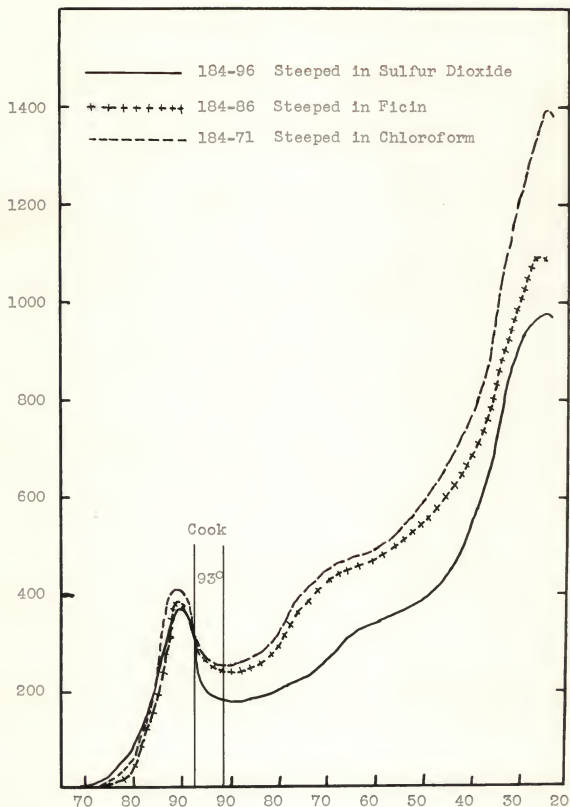


Fig. 2. Effect of three steepants on the viscosity of starch pastes. Steeped at 67° C.

Table 9. Correlation of paste characteristics with the steeping agent, temperature of steep, length of steep, and starch yields. Starch yields calculated on a dry basis.

Sample number	Steeping agent used	Temp. of steep : ° C.	Length of steep : hours	Yield of starch : percent	W <sub>1</sub> : hot peak	W <sub>2</sub> : cold peak	Waste : index	Gel : strength	Gel : index
Oven dried (70°)									
184- 2	chloroform	50	18	58.50	408	1552	3.31	1545	14.3
3	chloroform	50	18	61.50	372	1506	3.51	1215	- 7.0
5	chloroform	50	18	60.05	437	1365	3.13	1589	16.9
15	chloroform	50	18	61.94	414	1234	2.98	1285	4.1
Air dried (room temp.)									
184- 12	chloroform	50	18	59.64	423	1206	2.85	1586	31.3
15	chloroform	50	18	64.72	420	1255	2.99	1753	39.9
105	chloroform	37	48	62.50	474	1078	2.27	2392	121.9
71	chloroform	67	48	56.10	382	1398	3.66	2198	57.2
37	acrylonitrile	50	18	61.59	452	1110	2.48	1969	77.4

Table 9. (cont.)

Sample number	Steeping agent used	Temp. of steep : C.	Length of steep : hours	Yield of starch : percent	Air dried (room temp.)				W <sub>1</sub> hot peak	W <sub>2</sub> cold peak	Waste index	Gel strength index
184-44	ethylene	50	18	59.50	445	1189	2.67	2338	57.2			
81	versine	67	48	66.60	221	526	2.37	1200	129.0			
96	sulfur dioxide	67	48	66.60	368	975	2.65	2224	128.3			
102	sulfur dioxide	50	18	60.80	468	1031	2.20	2301	121.8			
100	lactic acid	50	48	55.80	456	950	2.08	2307	141.8			
110	sulphosalicylic acid	50	48	54.90	485	1050	2.33	2474	135.6			
104	pepsin	50	48	60.40	461	1072	2.33	2205	87.2			
84	ficin	67	48	74.33	410	1098	2.68	2363	115.2			
86	ficin	67	48	75.40	409	1097	2.68	2357	114.2			
99	ficin	50	48	73.60	436	1030	2.36	2425	135.3			
107	ficin	37	48	69.10	420	1242	2.96	2326	87.2			

Table 9. (concl.)

Sample number	Steeping agent used	Temp. of steep : ° C.	Length of steep : hours	Yield of starch : percent	W <sub>1</sub> : hot peak	W <sub>2</sub> : cold peak	Waste : index	Gel strength : index
Air dried (room temp.)								
184-108	ficin (.5 gm) sodium thio- sulfate (10 gm)	50	48	63.60	445	1033	2.32	2447 137.9
109	ficin (.5 gm) sodium thio- sulfate (1 gm)	50	48	69.60	437	1068	2.44	2147 101.0
114	ficin (.5 gm) cysteine hydro- chloride (2 gm)	50	48	71.50	440	994	2.26	2373 138.7
111	cysteine hydro- chloride	50	48	65.60	444	1043	2.35	2416 131.6
112	cysteine hydro- chloride (1 gm) acetic acid (2 cc)	50	48	64.30	452	953	2.11	2218 132.7
113	cysteine hydro- chloride (1 gm) lactic acid (2 cc)	50	48	68.50	441	974	2.21	2084 115.9

steeped at 50° C. The gel index, however, shows the most radical change: the higher temperature maintained in the oven had the tendency to cause a shortened paste. This is another illustration of the tendency of the starch granules to progress toward a more formalized structure when conditions permit.

Only a general, rough comparison could be drawn between the starch paste behavior of the various starches steeped in differing steep agents, as the content of protein and other adsorbed constituents probably are not comparable. Some general conclusions can be drawn from the data listed for starches obtained from differing steeps. It was noted that the standard Westland milo starch, milled by the method adopted as standard in this laboratory, had a hot peak of around 420, a cold peak of about 1250, paste index of around 3.0, a gel strength of approximately 1600 to 1800, and a gel index of from 30 to 45.

As was previously stated, the major effect, during processing, seemed to be caused by the temperature, but the effect of the steepant likewise may be pronounced. Versine was observed to have a very deleterious action on starch; it lowered the curve throughout the temperature cycle, and gave a lowered past index. This was probably the result again of the granules going toward a structure which requires more energy to gelatinize. The gel index in this case is somewhat above that obtained in the chloroform experiments; this shows that the length is somewhat increased by this steep. The results observed above are true to a greater or lesser degree for all acids employed in the steep. This was

readily noted from the data given for starches obtained from the steep in lactic acid, acetic acid, versine, sulfur dioxide, and sulphosalicylic acid. Ethylene and acrylonitrile both appeared to raise the  $W_1$  values over those obtained in the chloroform steep. The paste index remained approximately the same for these starches, but the gel index was somewhat increased. The ficin steeps gave values for  $W_1$  approximately the same as those obtained in the chloroform steeps, but the paste indices are somewhat decreased. The gel indices of the starches obtained in the ficin steeps were considerably higher than those present in starches from comparable chloroform steeps. This indicated that ficin tended to make a longer starch. Two of the experiments which were run using activators with a small amount of ficin, 184-108 and 184-114, possessed  $W_1$  values somewhat above those found with ficin alone, but their paste indices and gel indices were nearly the same as those obtained by the use of ficin alone in the steep. The gel index of the starch obtained in experiment No. 184-109, using one gram of sodium thiosulfate as an activator, is somewhat lower than those for the other two activator experiments; this may possibly be due to the adsorbed protein as the  $W_1$  and paste index values deviate little from those of the other two experiments.

The cysteine hydrochloride steep gave a  $W_1$  value above those obtained in the standard chloroform experiments, a paste index somewhat below, and a gel index far above the values noted for starches from the chloroform steep; thus it may be said to lower

the pasting capacity somewhat but greatly lengthen the starch paste. The last two experiments, 184-112 and 184-113, employing an acid steep prior to addition of cysteine hydrochloride, gave results similar to those of cysteine hydrochloride alone.

#### SUMMARY

Westland milo sorghum grits were wet milled by a standard procedure, in which chloroform was used as a steepant, to give a series of experiments to provide a basis for comparison of the effects on the starch yield and quality of variation of steeping conditions and steeping agents. It was found, using the standard milling procedure, that temperature of steep and length of steep had little effect on the yield of starch, but that the higher temperatures in the steep had a deleterious effect on the pasting qualities of the isolated starch.

Experiments using various non-enzymatic steeping agents gave no notably increased yields, though sulfur dioxide gave somewhat higher yields than did chloroform alone. Versine was noted to give a slightly higher yield of starch also, but it had an adverse effect on the starch pasting qualities. All of the acid steepants employed in these experiments gave a lowered pasting capacity and a longer starch paste when compared with those obtained from starches steeped in the standard chloroform solution. Diethylamine and triethylamine were noted to have an adverse effect on the starch yield.

The experiments using enzymes in the steep have shown ficin

to be the best of the steeping agents used. Experiments with this enzyme gave yields of nearly 90 percent of the total starch present in the grits. The yield using this enzyme was shown to be dependent upon the temperature of the steep and the amount of enzyme present in the steep solution. Pepsin proved to have no noticeable effect on the starch yield at the concentrations used in these experiments. The ficin steep was shown to produce a starch with better pasting characteristics than the original chloroform steep experiments. Experiments using two enzyme activators with a lesser amount of ficin proved cysteine hydrochloride to be an efficient agent in activating the enzyme. This activator gave yields which compare favorably with the yields obtained using a larger amount of ficin alone.

A mechanism was proposed for the action of the steeping agent on the protein involving two steps; the first being the unfolding of the protein to expose the site of the disulfide link, and the second being a metathetical reaction or an oxidation-reduction of the disulfide group, to form two sulfhydryl groups.

Attempts to duplicate this mechanism, using a preliminary steep in lactic or acetic acids, followed by addition of cysteine hydrochloride gave somewhat increased starch yields.

A feeding stuff analysis was taken on several samples and it was shown that the fiber content of the screenings tended to vary directly with the starch yield. It was also shown that the starch obtained in the high-yielding ficin experiment was relatively free of impurities.

Viscosity curves were recorded for many of the starches obtained in these experiments and were tabulated with the starch yields. It was shown that the starch pasting qualities were dependent upon both the temperature of the steep and the nature of the steeping agent employed.

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STEEPING STUDIES ON THE ENDOSPERM FRACTION  
OF MILO SORGHUM

by

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The steep reaction is of primary importance in the modern practice of wet milling because the starch yields, which are limited by the degree of softening of the protein in the steep, is the determining economic factor in this operation. The pasting qualities of the starch also are greatly affected by the conditions of the steep.

The primary purpose of this problem was directed at increasing the starch yields obtained from sorghum grits without causing deleterious effects on the starch granules during the steeping operation. The secondary objective was the establishment of a possible mechanism for the protein softening reaction in the steep.

The method of attack on this problem was an empirical study of the starch and by-products yields obtained from experiments utilizing various steepants under differing steeping conditions.

A method, standard with respect to this laboratory was adopted for the wet milling of 12 samples of grits. Chloroform was employed as a steepant in this standard series and was added in equal amounts to all subsequent experiments along with the steepant being investigated. This standard series was used as a basis for comparison of yields obtained varying the steepant while keeping the remainder of the process constant.

Experiments utilizing non-enzymatic steepants gave, with the exception of sulfur dioxide, no notably increased starch yields. Sulfur dioxide gave increased starch yields but yielded

starches with inhibited pasting characteristics. Deleterious effects on the starch paste characteristics and starch yields were noted for several of the other steepants. The quality of the starch was also shown to be dependent on the temperature of the steep and drying conditions.

Experiments with two enzymes, ficin and pepsin, present in the steep water were recorded. Ficin was noted to give a high yield of starch but pepsin was not effective. Ficin gave a yield of about 93 percent of the starch present in the grits.

A mechanism was proposed for the softening reaction in which there occurred two steps: the first being a distortion of the structure of the native protein, exposing the active sites present; the second was postulated to be a reaction of the disulfide links to form sulfhydryl groups.

Attempts to reproduce this mechanism by a preliminary acid steep, followed by the addition of cysteine hydrochloride, gave somewhat increased starch yields as compared with the standard chloroform. It was concluded that both cell permeability and the breaking of the disulfide link probably play a part in the mechanism of the steep reaction.